PLOIDY OF LIVING CLONES OF HUMAN SOMATIC CELLS DETERMINED BY MENSURATION AT METAPHASE

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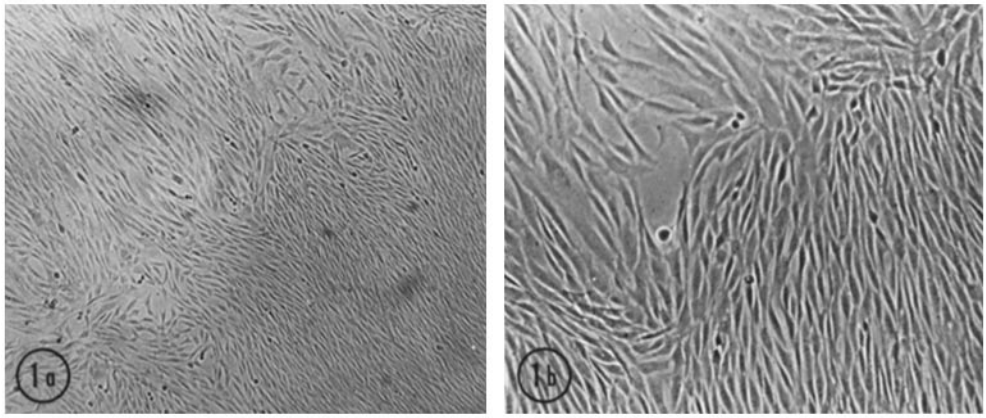
INTRODUCTION

Tetraploid clones have been useful for studies of somatic segregation (10), mutation (4), and the regulation of protein synthesis (14, 13, 12). With the availability of various heterochromatin markers, such material is also proving valuable for cytological studies of interphase (5). Finally, clones of cells with various ploidies should serve as very precise and convenient standards for flow microfluorometric assays of cell DNA (8, 1). We report here a rapid and simple technique for the diagnosis of ploidy in clones of somatic cells derived from human skin and amniotic fluid. The method employs objective measurements on small samples of living cells. Other methods, such as karyotyping and chemical assays, take much longer and require sacrifice of all or part of the cultures.

MATERIALS AND METHODS

Strain 73-4 was derived from the dermis of a 24-yr old female referred for cytogenetic studies because of reproductive failure Her karyotype was 46, XX. Cultures were examined at both early passages (about 10 cumulative population doublings) and middle passages (about 20 cumulative population doublings) (11). Strain 71-38 was a fibroblast-like culture derived from the skin of a 30-yr old normal male. Strain AC-16 became available after prenatal diagnosis at the 17th gestational week of a male fetus. General culture conditions were as previously described (11). Media was a modification of the Dulbecco-Vogt formulation (3) with 16% (vol/vol) heat-inactivated fetal calf serum. Cytochalasin B treatments (2 μg/ml for 36 h) for induction of tetraploidy (6) were carried out 6–8 h after dilute plating of 40–60 cells in 4 ml of media in 60-mm plastic Petri dishes. The cultures were then fed every third day. Between days 10 and 15, consecutive clones were analyzed by a two-step procedure: (*a*) four to 12 living mitotic cells were photographed at the stage of distinct equatorial alignment of chromosomes using a Nikon inverted microscope (model MS) with a 20× DLL objective and phase condenser. Measurements of the lengths of these metaphase plates were determined from projections of the photographic negatives before knowledge of cytogenetic analysis. The measurements were made as linear vectors transversely to the major spindle axes from mid-outer chromosome to mid-outer chromosome. (*b*) Clones were processed for chromosomal analysis by an *in situ* technique (9). Approximately 10–50 metaphase plates from each clone were examined. Five of these revealed mosaicism in the range of 10–30% diploidy: 70–90% tetraploidy. A similar analysis, with larger samples of metaphase mensurations, was carried out with the predominately diploid mass cultures and established passaged tetraploid clones from two of the strains.



FIGURES 1 *a* and *b* Contiguous diploid (lower right) and tetraploid (upper left) clones from strain 73–4. Phase contrast. (*a*), × 46; (*b*), × 154.

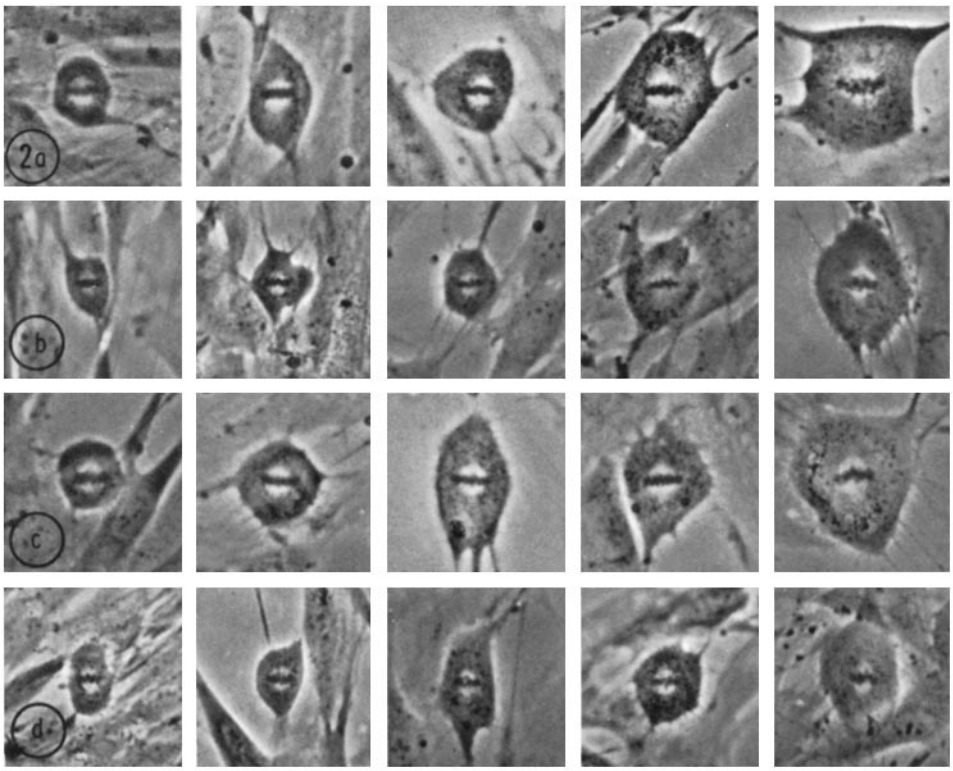


FIGURE 2 Series of metaphases (strain 71-38) illustrating constancy of lengths of equatorial plates for given ploidy despite variations in amounts of cytoplasm (increasing from left to right). Rows (*a*) and (*c*), tetraploid cultures; rows (*b*) and (*d*), diploid cultures. Phase contrast. × 675.

RESULTS AND DISCUSSION

Fig. 1 *a* and *b* shows areas of contiguous diploid and tetraploid clones of a fibroblast strain. A distinction on morphologic grounds was sometimes possible when the two clones were contiguous and at comparable stages of development. While the impression of increased size has been successfully employed in the use of certain rat fibroblast clones (13), we found it unreliable for human fibroblast-like cell lines, since there is considerable heterogeneity of cell size within and between typical clones. Turning our attention to the nuclear morphology of living cultures, it became apparent that a parameter which could be precisely measured at a brief and highly specific stage of the mitotic cell cycle was the length of the equatorial plate at metaphase (Fig. 2). Table I summarizes a series of such measurements derived from predominantly diploid mass cultures and cytogenetically proven tetraploid clones established from these cultures. The differences between the means

TABLE I

Mensurations of the Projected Lengths of the Equatorial Plates of Metaphase Cells from Diploid (D) Mass Cultures and Established Tetraploid (T) Clones Derived from Strains AC-16, 73-4, and 71-38

	No. of metaphases	Projected mean length	SD	*P*
		mm		
AC-16 (D)	83	9.9	1.8	<0.001
AC-16 (T)	78	12.3	1.7	
73-4 (D)	101	8.5	1.7	<0.001
73-4 (T)	57	11.4	1.8	
71-38 (D)	32	14.6*	1.9	<0.001
71-38 (T)	49	20.7*	2.1	

* For strain 71-38, a projection scale of 1 mm was equivalent to 0.4 μm of actual length; projection scales for other strains was as in Fig. 3.
SD = standard deviation.

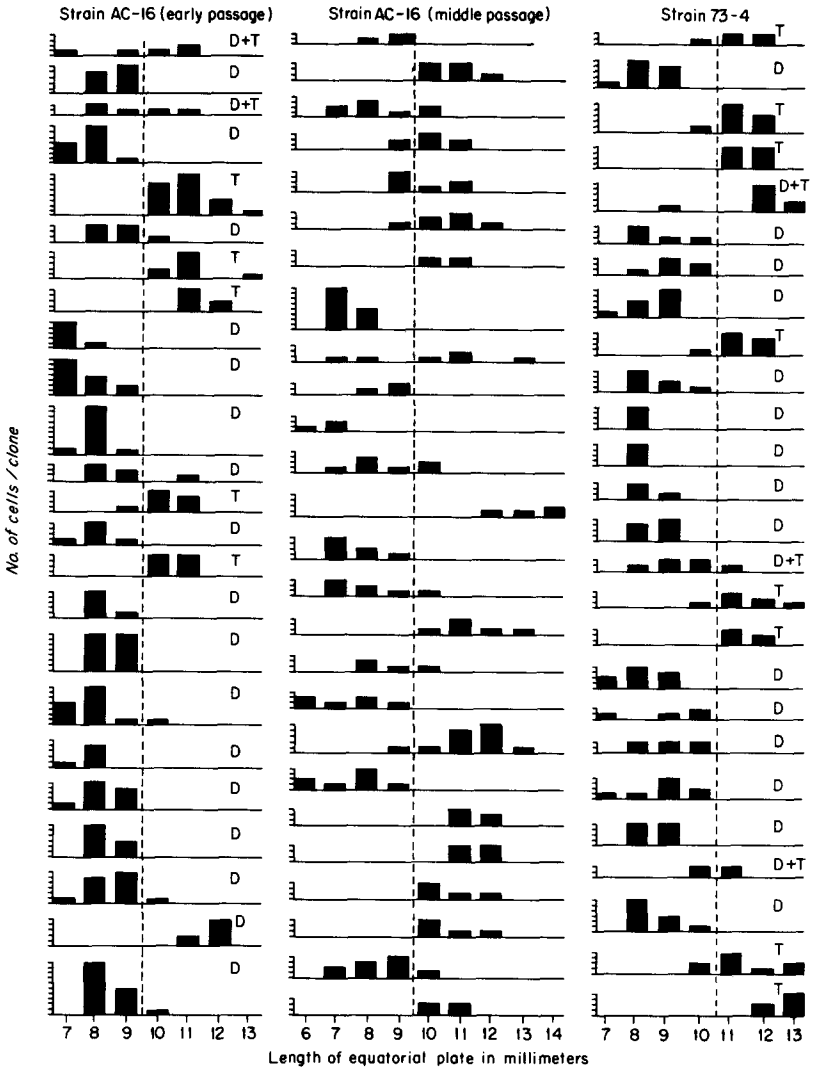


FIGURE 3 Mensuration of the equatorial plates of metaphase cells from consecutive clones of strains 73–4 and AC-16. The dotted lines indicate the optimal points of differentiation of the statistical distributions of the diploid and tetraploid sets. In the case of strain AC-16 (middle passage) the position of the line is presumptive, since cytogenetic studies were not done. Cytogenetically confirmed clones (strains AC-16, early passage and 73–4) are indicated by (*D*) diploid, (*T*) tetraploid, and (*D* + *T*) diploid-tetraploid mosaics. The abscissas represent projected lengths, 1 mm of which is equivalent to 0.7 μm of actual length.

of the two classes of material were highly significant ($P < 0.001$). We then sought to determine if it were possible to diagnose the ploidy of young emergent clones with small samples of such measurements. Of 50 clones (Fig. 3), 13 were classified as tetraploid, 32 as diploid, and five as presumptive mosaics. The independent cytogenetic studies of these 50 clones confirmed the diagnosis in all cases with the exception of a single presumptively mosaic clone, which appeared to be diploid. It is of interest that there may be some strain variation in that the distribution for the skin fibroblasts (strain 71–4) appears to be shifted to the right, as compared to strain AC-16, consistent with about a 17% increase in mean length of the equatorial plate. While it is conceivable that this may reflect intrinsic

differences in the genotype or, more probably, the cell type, methodological variation would have to be ruled out by a more extensive series of experiments. Fig. 3 also suggests that there is a broader distribution of metaphase mensurations among clones derived from "older" cultures (middle passage AC-16 vs. early passage AC-16) (11).

The method should be subject to further simplification by substituting direct micrometry for photography. It is comforting that, in this era of sophisticated instrumentation, modest extensions of the work of Flemming (2) and Jacobi (7) have proven useful.

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